

# A new gregarious parasitoid species, *Microplitis idreesae* (Hymenoptera, Braconidae, Microgastrinae) reared from *Mythimna* sp. (Lepidoptera, Noctuidae), with a key to the species of *Microplitis* in the Kingdom of Saudi Arabia

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## Abstract

A new species of parasitoid wasp (Braconidae, Microgastrinae) from the kingdom of Saudi Arabia (KSA) is described, *Microplitis idreesae* sp. nov. The genus is reported for the first time in the Eastern province of the KSA. This is the first host-parasitoid association for *Microplitis* documented in the country. The new species attacks *Mythimna* Ochsenheimer (Lepidoptera, Noctuidae). Natural history information is provided such as the association of males with females, geographical location, possible food plants, and details of wasp cocoons. In addition, a fragment of the mitochondrial cytochrome b gene is presented. A taxonomic key to the species of *Microplitis* reported from the KSA is provided. Characters of this new species and its affinities with the three previous species described from the KSA and four of the closely related to Palaearctic species are also discussed.

**Keywords**

Armyworm, biological control, host-parasitoid association, Middle East, taxonomy

**Introduction**

*Microplitis* Foerster, 1863 is a diverse and cosmopolitan genus within the braconid subfamily Microgastrinae. The name *Microplitis* word derives from the Greek prefix “micro” (meaning “small”) and “oplitēs” (meaning “armed”) and means small sword/weapon, referring to the generally short ovipositor present in the females (Foerster 1863; Fernandez-Triana et al. 2020). Currently, 192 species have been described (Fernandez-Triana et al. 2020). The Palaearctic (102 spp.), Oriental (40 spp.), and Nearctic (39 spp.) regions hold the great majority of the species followed by Australasian (25 spp.) region and to a lesser extent the Neotropical (11 spp.) and Afrotropical (1 spp.) regions (Fernandez-Triana et al. 2020). The Afrotropical and Neotropical regions are extremely understudied and there is potential to add new species. More than five hundred *Microplitis* species are estimated worldwide (Fernández-Triana et al. 2020).

*Microplitis* species are larval koinobiont endoparasitoids, meaning that their hosts continue to develop after being attacked. They are largely specialized in attacking two of the most recently derived superfamilies of Lepidoptera: Noctuoidea and Bombycoidea (Fernandez-Triana et al. 2015; Yu et al. 2016). As for the Middle East host records, 18 lepidopteran families have been reported (detailed host information from the species hosts can be found in Yu et al. 2016; Whitfield et al. 2022).

The Arabian Peninsula is the world’s largest peninsula and extraordinarily little is known regarding the Microgastrinae fauna (Fernandez-Triana and van Achterberg 2017). It is traditionally included in the Middle East region (Whitfield et al. 2022), and a total of 21 species in 12 genera have been reported in four countries of the Arabian Peninsula. Most of the species were described from Yemen (17 species in 11 genera) followed by the Kingdom of Saudi Arabia – KSA (five species in three genera), the United Arab Emirates – UAE (three species in three genera), and Oman (one species in one genus) (Fernandez-Triana and van Achterberg 2017; Whitfield et al. 2022).

Of the seven countries that comprise the Arabian Peninsula, including the southern portions of Iraq and Jordan, the KSA is the largest in terms of area. In 2017, even though one morphospecies (*Microplitis* sp. 6) was reported from the KSA, no formal species description is available (Fernandez-Triana and van Achterberg 2017). Later in 2020, three *Microplitis* species were described, all authored by Ghramh and Ahmad 2020: *M. faifaicus* (Faifa town, Asir mountain range, Jazan Province), *M. khamisicus* (Khamis Mushyat city, Asir Province), and *M. tihamicus* (Abha city, Almanaf, Asir Province). All the specimens included in the previous study were collected with Malaise traps set up in the southwestern corner of the country. Only females were collected, and information about herbivore hosts and food plants was not available at that time (Ghramh et al. 2020). To date, the KSA is the only country in the Arabian Peninsula where described species of *Microplitis* have been reported.

The aim of this paper is to describe and illustrate one new species of *Microplitis* from the KSA. The specimens were reared from an armyworm caterpillar at the campus of King Faisal University (KFU), in Al-Ahsa region of the KSA. An identification key for all the valid *Microplitis* species reported in the KSA is provided. Additional information such as geographical location, association of males with females, potential food plants, details of wasp cocoons, and DNA sequence for a fragment of the mitochondrial cytochrome b (Cyt-b) gene are also provided.

## Materials and methods

### Collecting site and rearing

Hofuf is the major urban city in the Al-Ahsa Oasis, in the Easter Province of the KSA. Al-Ahsa is the largest oasis in the world and is one of the two most important oases in the Arabian Peninsula; the other is Al Ain in the UAE, on the border with Oman. The climate of Hofuf is a hot desert (*BWh*) (Köppen 2022), the summers are extremely hot and long, while the winters are mild and short (National Meteorology and Environment Center 2015). It is known for being one of the largest date palm (*Phoenix dactylifera*, Arecales: Arecaceae) producers in the world. The crops thrive on salty soils that are fed from an underground aquifer and irrigated by the flow of many artesian springs, which allows agriculture all year round in a region that is otherwise a sandy desert (Mohammed et al. 2022).

On the 18<sup>th</sup> of December 2020, a caterpillar (3cm in length, Fig. 1A) was found crawling on a botanic wall, two meters away from the grass on the student residential housing, inside the campus of KFU, Hofuf city. The caterpillar was carefully removed and placed inside a plastic container and transported to one of the residential rooms. It was later observed that 15 silk cocoons were spun beside the caterpillar (Fig. 1B). To avoid the effects of decomposition of the caterpillar and use it for further identification, the pupae with cocoons already hardened were gently detached from the back of the caterpillar, placed in a clean dry container, and divided into two groups to study the effect of different incubation conditions on the duration of pupation. The first group of cocoons (n=8) was incubated at room temperature (22 °C), 20–30% relative humidity (RH), and 10.5 light (L):13.5 dark (D) photoperiod in a polypropylene tube with a loose cap for aeration (Fig. 1C). The second group of cocoons (n=7) was incubated at 29 °C and 60% RH in an environmental chamber (Shell Lab, USA) in complete darkness until the emergence of the adult parasitoids. After adult emergence, all were kept at -20 °C and later point mounted or kept in the freezer for molecular analysis. The time it took for the pupae to become adult wasps was recorded for both groups. The still-living caterpillar was kept in a plastic vial in the environmental chamber and observed daily until its death. The dead caterpillar was photographed immediately (Fig. 2), and only then preserved at 70% ethanol.



**Figure 1.** Lepidoptera host (*Mythimna* sp., Noctuidae: Hadeninae, Leucaniini) and parasitoid wasps (cocoons and adults of *Microplitis idreesae* sp. nov.) **A** living caterpillar of *Mythimna* sp. in its last instar larval **B** wasp cocoons attached to the back and sides of the living caterpillar **C** Adult wasps emerged after their incubation at room temperature.



**Figure 2.** Dead caterpillar of Lepidoptera host (*Mythimna* sp., Noctuidae: Hadeninae, Leucaniini) showing exit holes (black circles) from which larvae of *Microplitis idreesae* sp. nov. have emerged **A** ventral side **B** lateral side **C** dorsal side.

## Taxonomic identifications

The caterpillar identification was performed by Dr. Steven Passoa from the United States Department of Agriculture, Animal and Plant Health Inspection Service, and Plant Protection and Quarantine Department in the United States. The caterpillar was not picked up on a specific plant and for this reason, the plants surrounding the place

where the caterpillar was collected were identified following Chaudhary and Akram (1987) as indications of possible food source.

As for the parasitoid wasp, initial identification at the genus level follows Whitfield (1997). Original *Microplitis* species descriptions from the KSA (Ghramh et al. 2020) and Palaearctic *Microplitis* keys and papers (Nixon 1970; Papp 1984; Shaw 2012) were also consulted. The geographic distribution of the genus *Microplitis* was obtained from published data (Fernandez-Triana and van Achterberg 2017; Fernandez-Triana et al. 2020; Whitfield et al. 2022), and the elevation data from the previously collected *Microplitis* species in the KSA was retrieved by using: <https://www.advancedconverter.com>. Terminology for surface sculpturing follows Harris (1979), wing venation follows van Achterberg (1993), and morphology follows Mason (1981), Austin and Dangerfield (1992), Sharkey and Wharton (1997), and Whitfield (1997). Additionally, morphological terms used recently to refer to structures mainly in the scutellum and the metanotum follows Arias-Penna et al. (2019 –figs 2 and 3 in pp. 27 and 31, respectively). All measurements are expressed in mm. Morphological terms and their abbreviations used within the text are: OOL = ocular ocellar line (the shortest distance between lateral ocellus and adjacent compound eye margin), POL = posterior ocellar line (the shortest distance between the lateral ocelli); S = metasomal sternum: S1 = sternum 1, S2 = sternum 2 and so on; T = metasomal tergum: T1 = tergum 1, T2 = tergum 2 and so on.

## Digital imaging

Digital photos were obtained using a Canon EOS 5DS R digital camera (Canon, Inc. Japan) with an affixed Canon MP-E 65mm f/2.8 1-5x Macro lens. The light was emitted from two fixed monolight sources (Interfit S1a 500Ws HSS TTL AC Powered Monolight). Specimen pictures were taken using an 18% gray background. The camera was mounted on an automated WeMacro™ rail to create a series of partially focused images, that were exported to Helicon Focus™ version 8.0.2. (<http://www.heliconsoft.com>) to produce a focused image. Further processing of the final images was done using Microsoft Photos™.

Images were also obtained by a Scanning Electron Microscope (SEM). Wings were not removed, and no pre-cleaning procedure was done before SEM. The wasps were sputter-coated in gold (Spi-Module Sputter Coater, UK), and photographed using a JSM 5200 electron probe microanalyzer (JEOL, Japan) at the Department of Microbiology, College of Veterinary Medicine at KFU (KFU-Vet).

The total number of adults as well as the number of females and males of the emerged parasitoids were reported. All the material, including the type specimens, are deposited in the Insect Collection of the Entomology Laboratory at KFU-Vet, KSA, under Item No: PW (Parasitoid Wasp): 12-2020, *Microplitis idreesae* reference items 3: females & 3: males.

## DNA extraction

DNA from one adult female wasp was extracted as adapted from the method of Dellaporta et al. (1983). Briefly, the wasp was manually ground in a 1.5 ml

microcentrifuge tube containing 50 $\mu$ L extraction buffer (100 mM Tris-HCl, 500 mM NaCl, 10 mM  $\beta$ -mercaptoethanol, 50 mM EDTA). The mixture was then incubated in a heating block set at 65 °C for 10 min. 10 $\mu$ L of 5M potassium acetate together with 50 $\mu$ L of phenol/chloroform/isoamyl alcohol (25:24:1) were then added. The new mixture was cooled by keeping the tube on ice for 10 min, followed by centrifugation at 10k rpm for 20 min. The supernatant was then transferred to a new tube and isopropanol of equal volume was added. This mixture was then incubated at -20 °C for 10 min, and the pellet was obtained by centrifugation at 10k rpm for 10 min. The supernatant was discarded, and the lid of the tube was left for air drying. The pellet containing DNA was then washed with 70% ethanol and eluted in sterile deionized distilled water (ddH<sub>2</sub>O).

### DNA amplification condition and sequencing

A fragment (381 bp) of the mitochondrial cytochrome b (Cyt-b) gene was amplified using previously reported primers: Cyt-b forward primer: TATGTACTACCATGAG-GACAAATATC, reverse primer: ATTACACCTCCTAATTATTAGGAAT (Simon et al. 1994). DNA amplification was performed using a high-fidelity polymerase enzyme and the accompanying PCR master mix according to the manufacturer's conditions (Accu Taq™ LA DNA polymerase, Sigma-Aldrich, USA). The PCR reaction was conducted in the thermocycler (Applied Biosystems™ Veriti™, USA), with initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 1 min, 48 °C for 1 min, and 72 °C for 1 min, and the final extension was 72 °C for 7 min. The PCR product was visualized in a 1.5% agarose gel stained with EZView Stain (Biomatik, Wilmington, USA). Amplicons were purified using a commercial kit according to the manufacturer's protocol (QIAquick, QIAGEN, Valencia, CA, USA). Sequences were generated via Sanger cycle-sequencing on both directions using the same primers (Macrogen, South Korea). The obtained sequences were assembled and edited using MEGA11 (Tamura et al. 2021). The partial nucleotide sequence of the Cyt-b gene was uploaded to GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>).

## Results

### Taxonomy

#### Key to the species of *Microplitis* Foerster reported from the Kingdom of Saudi Arabia

1	Notauli weakly impressed, indicated only by narrow depressions postero-laterally (figs 1A, 2A, Ghramh et al. 2020, pp. 3, 4).....	2
–	Notauli strongly impressed and coarsely sculptured (Fig. 3B, D) .....	3

2(1) Fore wing with triangular areolet (fig. 1C, Ghamr et al. 2020, p. 3); vein 1SR+M almost straight (fig. 1C, Ghamr et al. 2020, p. 3) .....  
..... ***M. tihamicus* Ghamr & Ahmad**

– Fore wing with quadrangular areolet (fig. 2C, Ghamr et al. 2020, p. 4); vein 1SR+M slightly curved downwards (fig. 2C, Ghamr et al. 2020, p. 4) .....  
..... ***M. faifaicus* Ghamr & Ahmad**

3(1) T1 with a median knob posteriorly (fig. 3B, Ghamr et al. 2020, p. 6); notauli strongly impressed throughout and meeting posteriorly but not reaching scutoscutellar sulcus (fig. 3A, Ghamr et al. 2020, p. 6); fore wing with vein 2-SR+M longer than vein 2-M (fig. 3C, Ghamr et al. 2020, p. 6); medial furrow of mesoscutum complete (fig. 3A, Ghamr et al. 2020, p. 6) .....  
..... ***M. khamisicus* Ghamr & Ahmad**

– T1 slightly elevated and rounded posteriorly (Fig. 3B, E); notauli strongly impressed anteriorly but indistinct posteriorly (Fig. 3B, D); fore wing with vein 2-SR+M as long as vein 2-M (Fig. 3J); medial furrow of mesoscutum incomplete, distinct only anteriorly (Fig. 3B, D) ..... ***M. idreesae* sp. nov.**

## Species description

### Genus *Microplitis* Foerster, 1863

*Microplitis* Foerster, 1863: 245. Type species: *Microgaster sordipes* Nees, 1834, by original designation.

**Diagnosis.** Fore wing usually with a large areolet; mesopleuron with epicnemial carina absence; propodeum with reticulated sculpture and often with a strong median longitudinal carina; T1 with a median longitudinal sulcus for most of its length, T2 and T3 unsculptured and separated by a weak suture; ovipositor short; hind coxa small, not surpassing T2, usually not surpassing T1; hind tibial spurs usually shorter than half length of first hind tarsomere; mesoscutum with notauli variable, ranging from weakly impressed (virtually absent indicated by indentations at anterior margin of mesoscutum or by pair of depressions postero-medially) to strongly impressed and coarsely sculptured (Nixon 1965; Mason 1981; Austin and Dangerfield 1992, 1993; Ranjith et al. 2015; Fernandez-Triana and van Achterberg 2017; Fernandez-Triana and Boudreault 2018).

**Comments.** After its establishment, the genus *Microplitis* has been beset by two problems: taxonomic and nomenclatural instability and an appreciable deficiency of distribution data (Fernandez-Triana et al. 2020). *Microgaster* Latreille, was the first genus of Microgastrinae to be described and is the basis for the subfamily name (Latreille 1804, Mason 1981, Fernandez-Triana et al. 2020). Some species that were described as *Microgaster* turned out to belong to the group we now call *Microplitis*. As for the associated geographic data, those reports have either been questioned or are scarce for the vast majority of the species (Fernandez-Triana et al. 2020).

*Microplitis* is part of a well-defined but informal group of eight genera that is probably monophyletic (Fernandez-Triana and Boudreault 2018; Fernandez-Triana et al. 2020). The other seven genera are: *Alloplitis* Nixon, *Gilbertnixonius* Fernandez-Triana, *Jenopappius* Fernandez-Triana, *Philoplitis* Nixon, *Silvaspinosus* Fernandez-Triana, *Snellenius* Westwood, and *Tobleronius* Fernandez-Triana (Fernandez-Triana and Boudreault 2018; Fernandez-Triana et al. 2020). Within this group of genera, *Microplitis* most resembles *Snellenius*, and the two genera have been considered closely related.

***Microplitis idreesae* Arias-Penna & Al-Sabi, sp. nov.**

<https://zoobank.org/34DEAC3F-4B21-4A3C-94AC-7376DB57A635>

Figs 3A–J, 4A–K

**Type material. Holotype.** THE KINGDOM OF SAUDI ARABIA. 1 Female; the Eastern Province, Al-Ahsa Oasis, Hofuf, King Faisal University student housing; 25°20'34.1412"N, 49°36'6.2316"E; 154m; 18.xii.2020; Nabila Idrees leg.; reared on undetermined species of *Mythimna* (Lepidoptera: Noctuidae); caterpillar collected from the wall of the housing compound at KFU; cocoons formed on 19.xii.2020; adults emerged on 27.xii.2020, 29.xii.2020, and 30.xii.2020; PW: 12-2020; (KFU-Vet).

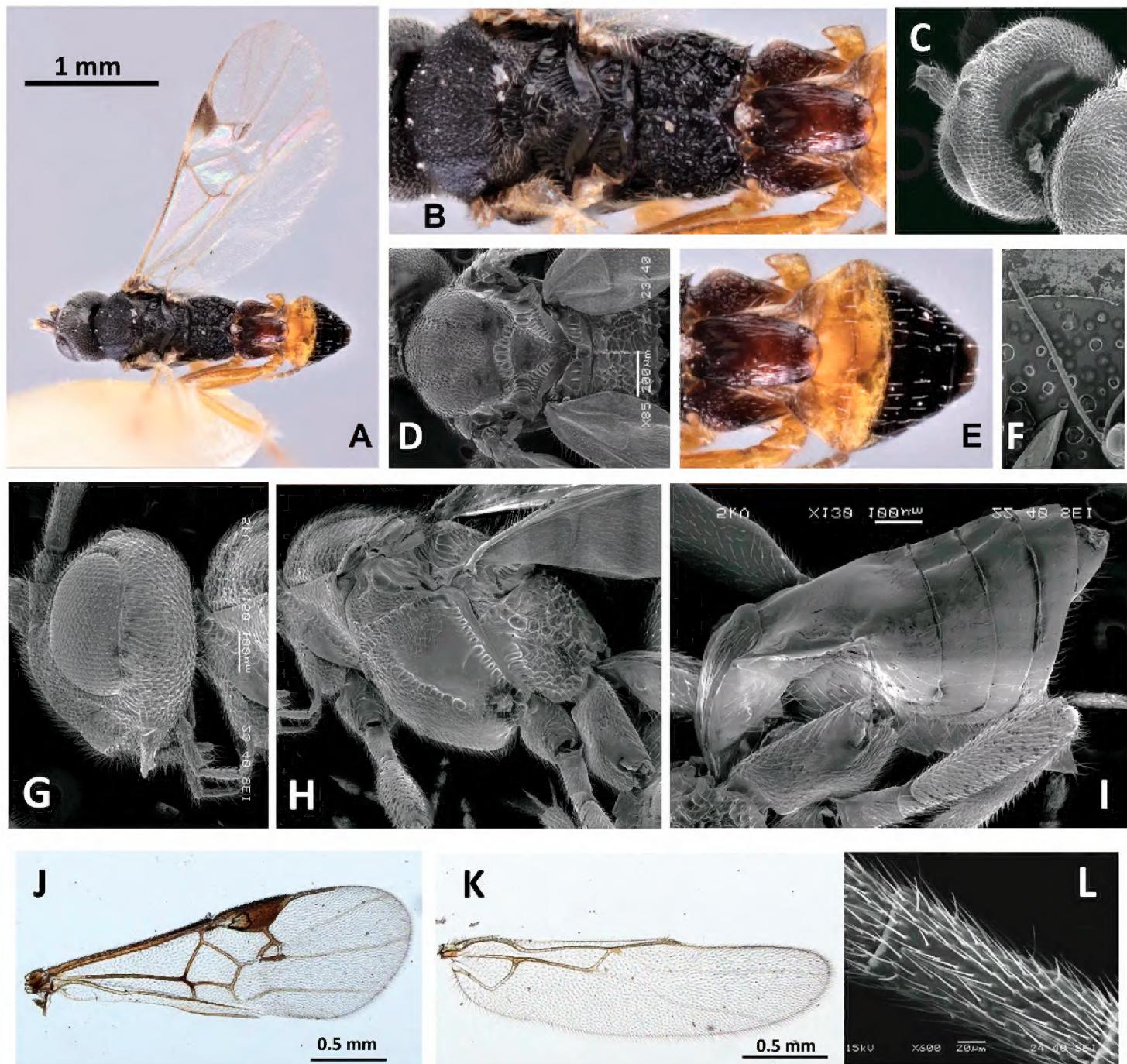
**Paratypes.** 5 (2 females, 3 males); same data as for holotype; (KFU-Vet).

**Diagnosis.** T1 slightly elevated and rounded posteriorly; fore wing with quadrangular areolet and vein  $r$  straight; notauli strongly impressed anteriorly but disappearing gradually as they approach the scutoscutellar sulcus.

**Description. Female.** Body length (head to apex of metasoma): 2.6, fore wing length: 2.45, antenna length: 3.36. Body length in females varies between 2.6 to 2.9.

**Colour** (Fig. 3 A, B, E). General body coloration black except for scape, pedicel, first ten proximal antennal flagellomeres, labrum, mandibles, maxillary and labial palps, and tegula dark yellow-brown. All legs dark yellow-brown except: all claws dark brown and hind coxae with basal third dark brown, second third black, and distal third yellow-brown. T1 dark brown-black, posteriorly with a dark yellow-brown area, lateral ends of T1 dark brown; T2 completely dark yellow-brown; T3 with anterior half dark yellow-brown and posterior half black; T4 and following completely black. In lateral view, T1–T2 completely dark yellow-brown; T3 with anterior half dark yellow-brown and posterior half black, T4 and following completely black. S1–S2 dark yellow-brown, S3 with anterior half dark yellow-brown and posterior half black, and hypopygium brown.

**Head** (Figs 3A, C, G). In frontal view, rounded with pubescence long and moderately dense. Proximal thirteen antennal flagellomeres longer than wide (0.18:0.06), last antennal flagellomere pointed and longer than penultimate (0.16:0.05, 0.12:0.05), all antennal flagellomeres setose, antenna longer than body (3.36, 2.6); scrobes shallow. Face rounded with dense fine punctations, interspaces wavy, and longitudinal median carina incomplete, visible only at the anterior third. Fronto-clypeal suture absent. Temple wide, punctate, and interspaces wavy. Inner eye margins diverge slightly at scrobes. POL shorter than OOL (0.10, 0.12). Malar suture present but long and



**Figure 3.** *Microplitis idreesae* sp. nov. female **A** habitus **B** mesosoma and T1, dorsal view **C, G** head **C** dorsolateral view **G** lateral view **D, H** mesosoma **D** dorsal view **H** lateral view **E, I** metasoma **E** dorsal view **I** lateral view **F, L** antenna **F** antenna **L** flagellomere **J, K** wings **J** fore **K** hind.

moderately dense pubescence makes its observation difficult. Median area between lateral ocelli with a depression. Vertex, rounded in lateral view, narrow in dorsal view.

**Mesosoma** (Figs 3A, B, D, H). Mesosoma dorsoventrally convex, length: 1.4. Mesoscutum anteriorly convex and posteriorly flat with punctuation distinct anteriorly, satiny posteriorly, and interspaces wavy/lacunose; medial furrow of mesoscutum incomplete, distinct only anteriorly. Notauli distinct anteriorly but disappearing gradually as they approach the scutoscutellar sulcus. Scutellum long and slender, posteriorly sloped and fused with medioposterior band of scutellum, scutellar punctuation fine scattered throughout, in profile scutellum flat and on the same plane as mesoscutum, phragma of the scutellum slightly visible; medioposterior band of scutellum sculptured and overlapping slightly the medioanterior pit of metanotum; axillary trough of scutellum demilune and dorsal axillary trough of scutellum groove with complete undulate/re-

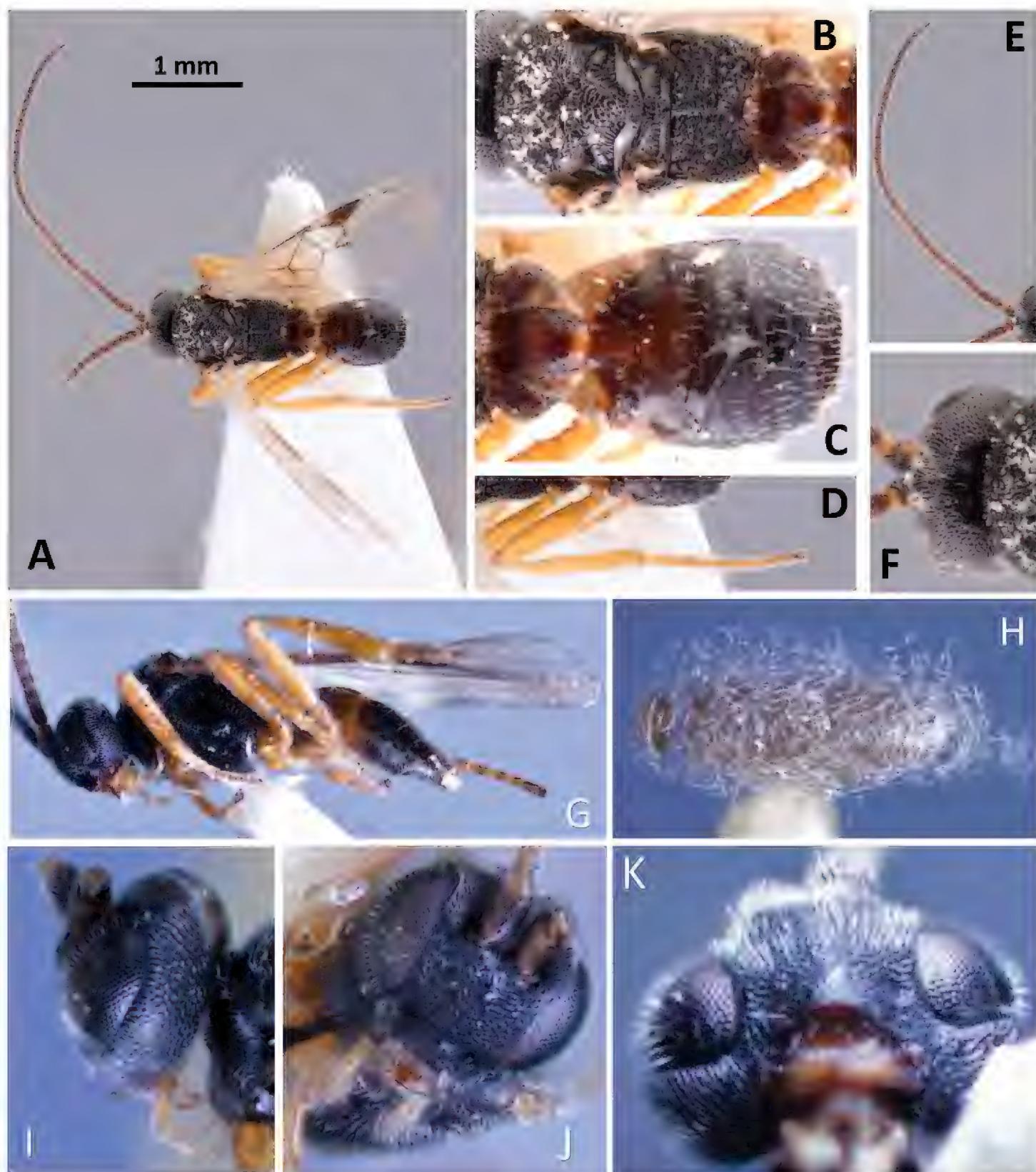
ticulate carinae. Scutoscutellar sulcus markedly bowed with seven irregular and deep foveae, the two middle ones larger than the others, area just behind scutoscutellar sulcus smooth, shiny, and nearly at the same level as mesoscutum (flat). Anterior furrow of metanotum with setiferous lobes and not as well delineated as posterior furrow which is thick and smooth; medioanterior pit of metanotum elongated with a complete transverse carina in its third posterior, overlapping completely the medioposterior band of metanotum which is difficult to differentiate; axillary trough metanotum with few incomplete parallel carinae. Propodeum with a distinct median longitudinal carina and areolate rugose sculpturing covering its entire surface; propodeal spiracles surrounded by carina; nucha ringed by radiating carinae. In lateral view, upper pronotum with imbricate sculpture throughout, centrally with a distinct furrow of deep irregular foveae, and lower pronotum with two types of sculpture, anterior half imbricate and posterior half smooth. Propleuron finely sculptured. Metasternum convex. Mesopleuron convex, lower mesopleuron finely imbricate, centrally smooth, dorsal margin with a distinct row of foveae forming an L-shape inverted, precoxal sulcus crenulate. Epicnemial ridge truncate-pyramid shape, anteriorly convex, posteriorly truncate.

**Legs** (Fig. 3A, B). Ventral margin of fore telotarsus entire, with a tiny straight seta, fore telotarsus basally narrow and apically wide, and longer than the fourth tarsomere (0.14, 0.08). Hind coxa finely punctate throughout, without outer depression; inner spur of hind tibia slightly longer than outer spur (0.13, 0.12); entire surface of hind tibia with dense strong spines uniform by color and length; hind telotarsus longer than the fourth tarsomere (0.17, 0.09); hind femur length:wide (0.70:0.17), hind tibia length (0.42), hind basitarsus length (0.13).

**Wings** (Fig. 3J, K). Fore wing: length 2.45; stigma dark brown but paler at base; quadrangular areolet, vein r-m spectral; vein 3-SR shorter than vein r-m, both veins forming an angle at their junction; vein 2-SR curved and longer than 3-SR; vein 2-M straight and swollen; r vein straight; 1-R1 length 0.58; vein 1-M straight; vein 1-SR+M slightly curved; vein 2-SR+M spectral; vein 3CU1 tubular; vein CU1a mostly spectral but a small anterior portion absent; vein CU1b absent; vein 2-1A tubular; vein cu-a slightly curved, reaching the edge of 1-1A vein, anterior half tubular, and posterior half spectral. Hind wing (length 2.0) with vannal lobe narrow, convex, with long setae.

**Metasoma** (Fig. 3A, E, I). T1 virtually parallel-sided (barrel-shaped) over most of its length but narrowing over the posterior 1/3 (length 0.41; maximum width 0.2; minimum width 0.13), distally rounded, slightly elevated medially, almost nitid with scarce sculpturing laterally and scattered pubescence in the posterior third. Median area on T2 polished, truncate-trapezoidal, slightly wider than longer (length 0.16, maximum width 0.17; minimum width 0.12); lateral grooves delimiting the median area clearly defined and not reaching the posterior edge (length median area 0.17, length T2 0.2); T3 smooth, as long as T2 (0.2, 0.2). Pubescence on the hypopygium scattered.

**Cocoons** (Fig. 4H). Cocoons are oval with light olive drab coloration, lacking any kind of remarkable ornament, and the silk fibers looking disordered and fluffy. Cocoons are located both on the anterior part of the back and lateral sides of the alive host caterpillar.



**Figure 4.** *Microplitis idreesae* sp. nov. male **A, G** habitus **A** dorsal view **G** lateral view **B** mesosoma and T1, dorsal view **C** metasoma, dorsal view **D** legs **E** antenna **F, I–K** head **F** dorsal view **I** lateral view **J** frontal view **K** ventral view **H** cocoon.

**Male (Fig. 4A–K).** Similar to female except T2 with lateral areas dark yellow-brown and T3 completely dark brown. Body length varies between 2.5 to 2.8.

**Etymology.** This species is named in honor of Nabila Rayed Nashaat Idrees who found the infested caterpillar. She is a bachelor student from the College of Veterinary Medicine at the King Faisal University, Al-Ahsa, KSA.

**Distribution.** The Kingdom of Saudi Arabia, Eastern Province, Hofuf.

**Biology.** Gregarious larval endoparasitoid wasp. Essentially all but one of the larvae successfully spun their cocoons (15 out of 16), out of which emerged 8 females and 7 male adults. The adults obtained from pupae incubated at room temperature (n=8) eclosed on the eighth day after pupation, whereas those incubated in the environmental chamber (n=7) took one to two days longer to emerge. It is worth mentioning that in nature, eclosion is tied to both internal physiological processes and externally received cues (e.g., evaporative cooling, heat retention by moist litter, –Janzen et al. 2003). This may suggest the adaptation of the wasps to different environmental conditions and suggest the existence of different habits related to the ambient conditions.

**Hosts** (Figs 1, 2). Undetermined species of the oriental armyworm *Mythimna* Ochsenheimer (Noctuidae: Hadeninae, Leucaniini). The living caterpillar was collected in the fifth instar. It took four days for *Mythimna* to die after the cocoons were detached from its body. In the living *Mythimna* caterpillar, the cocoons of *M. idreesae* were more clustered in the central part of the caterpillar body forming a dorsal band and a few were in the latero-posterior side (Fig. 1B). In the dead caterpillar, the holes were observed in the dorsal, ventral, and lateral sides of the body (Fig. 2).

Three potential food plant species were identified in the vicinity of the collected caterpillar, *Ipomoea pes-caprae* (Convolvulaceae, bay-hops), *Euphorbia serpens* (Euphorbiaceae, matted sandmat), and *Cynodon dactylon* (Poaceae, bermudagrass).

**Molecular data.** The partial nucleotide sequence of Cyt-b gene (381 bp) is available in the GenBank database, accession number: [OP485682](#).

**Remarks.** Morphological and distributional data that allow the separation of all the *Microplitis* species reported in the KSA is listed in Table 1.

*Microplitis idreesae* is closely related to Palaeartic species that exhibit the T1 barrel-shaped with scarce sculpturing; the legs with a light coloration (at least moderately, as it can be variable) except the hind coxa; and the fore wing with pterostigma bicoloured (dark with a pale basal spot). Considering this, four *Microplitis* species look similar to *M. idreesae*. In alphabetic order, these are *M. albipennis* Abdinbekova, *M. hispalensis* Marshall, *M. mandibularis* Thomson, and *M. spectabilis* Haliday. Similarities and differences between *M. idreesae* from these species are listed below.

***Microplitis idreesae* and *M. albipennis*.** In both species the wings are hyaline, the fore wing with the 1-R1 vein short, half as long as the pterostigma, the position of the r vein is oblique concerning the pterostigma, and the r vein is only somewhat shorter than the 2-SR vein.

*Microplitis idreesae* can be separated from *M. albipennis* by the following characters: 1) length of the T1: in *M. albipennis* is 1.6–1.7 times as long as broad, whereas in *M. idreesae* is 2.0 times as long as broad; 2) the colour on the tegula: in *M. albipennis* is black, whereas in *M. idreesae* is dark yellow-brown; 3) in the hind wings, the length of the 1-SR and 2M veins: in *M. albipennis* the 2-M vein hardly is 1.5 times longer than 1-SR, whereas in *M. idreesae* the 2-M vein is 1.7 times longer than 1-SR.

*Microplitis albipennis* has been reported in Azerbaijan, Hungary, Mongolia, Poland, Russia, and Turkey (Fernandez-Triana et al. 2020); and its lifestyle is unknown.

**Table 1.** All *Microplitis* species from the Kingdom of Saudi Arabia displaying their morphological differences and distributional data.

	<i>M. faifaicus</i> Ghramh & Ahmad, 2020	<i>M. idreesae</i> Arias-Penna & Al-Sabi, sp. nov.	<i>M. khamisicus</i> Ghramh & Ahmad, 2020	<i>M. tihamicus</i> Ghramh & Ahmad, 2020
Body size	2.5 mm	2.59 mm	3.0–3.1 mm	2.1–2.2 mm
Colour on body	Body generally black, T1 brown, palps and hind tibial spurs yellow, legs yellow except hind coxae	Body generally black, all legs dark yellow-brown except all claws dark brown and hind coxae with basal third dark brown, second third black, and distal third yellow-brown, T2 and anterior half of T3 yellow-brown	Body entirely dark brown to black excluding all legs and laterotergites dorsolaterally yellowish	Body entirely dark brown to black excluding yellowish legs
Colour on wings	Infuscate	Hyaline	Infuscate	Hyaline but slightly infuscate distally
Areolet shape	Quadrangular	Quadrangular	Quadrangular	Triangular
Shape of T1 posteriorly	Rounded/convex	Slightly elevated, rounded/convex	With a median knob	Truncate
Notauli	Weakly impressed, indicated only by narrow depressions postero-laterally	Well defined anteriorly but indistinct posteriorly	Well defined throughout	Weakly impressed, indicated only by narrow depressions postero-laterally
Medial furrow of mesoscutum	Absent	Present and incomplete	Present and complete	Absent
Setae in head and mesosoma	Moderately to sparsely setose	Moderately to sparsely setose	Densely setose	Moderately to sparsely setose
Distribution in the KSA	Faifa (Jazan Province)	Hofuf (Eastern Province)	Khamis Mushyat (Asir Province)	Abha, Almanaf (Asir Province)
Elevation (m)	906	154	1988	2226
<i>Microplitis</i> species to which it most resembles	<i>M. hova</i> (Granger, 1949) from Madagascar	<i>M. albipennis</i> (Abdinbekova, 1969), <i>M. hispalensis</i> (Marshall 1898), <i>M. mandibularis</i> (Thomson 1895), and <i>M. spectabilis</i> (Haliday, 1834) from the Palaeartic	<i>M. bambusanus</i> (de Saeger, 1944) from Congo and Rwanda	<i>M. isis</i> (de Saeger, 1944) from Congo

Black/white line drawings of some structures are available on Papp 1984 (fig. 73, p. 123; figs 146, 147, p. 129).

***Microplitis idreesae* and *M. hispalensis*.** In both species the first antennal flagellomere is thrice longer than broad, further flagellomeres gradually shorten so that the penultimate is twice longer than broad; the precoxal sulcus is crenulate; and with gregarious lifestyle.

*Microplitis idreesae* can be separated from *M. hispalensis* by the following characters: 1) female body size: in *M. hispalensis* the length is 3 mm, whereas in *M. idreesae* is 2.6–2.9 mm; 2) position of the r vein concerning the pterostigma: in *M. hispalensis* the vein r is perpendicular to the pterostigma, whereas in *M. idreesae* the vein r is oblique to the pterostigma; 3) colour on the body: in *M. hispalensis* is completely black, whereas in *M. idreesae*, females with T2 completely pale and T3 bicoloured (half anterior pale, half posterior dark), contrasting with the colour on the males, where the pale colora-

tion is confined only to a small area, anterior corners of T2; 4) colour on the wings: in *M. hispalensis* is weakly smoky (famous), whereas in *M. idreesae* is hyaline; 5) colour on the legs: in *M. hispalensis* is black although, in males, the legs show a light pattern and more infuscation, whereas in *M. idreesae* the legs are completely dark yellow-brown, except the hind coxa with basal third dark brown, second third black, and distal third yellow-brown; 6) and in *M. hispalensis* the antenna is as long as the body, while in *M. idreesae* is antenna is longer than the body.

*Microplitis hispalensis* has been reported in France and Spain (Fernandez-Triana et al. 2020). Black/white line drawings of some structures are available on Papp 1984 (figs 141–144, p. 129).

***Microplitis idreesae* and *M. mandibularis*.** In both species, the fore and middle coxae are entirely yellow, and the hind coxa is frequently splashed with yellow, and with gregarious lifestyle.

*Microplitis idreesae* can be separated from *M. mandibularis* by the following characters: 1) colour on T2 and T3: in *M. mandibularis*, sometimes the females display the T2 and the T3 very marked with yellow, whereas in *M. idreesae*, the females with the T2 completely pale and the T3 bicoloured (half anterior pale, half posterior dark); 2) colour on the male antennal flagellomeres: in *M. mandibularis* they are pale throughout though this is sometimes more obvious on the underside, whereas in *M. idreesae* the pale colouration (yellow-brown) is clear in the first eight proximal antennal flagellomeres and gets gradually darker in the next two flagellomeres (9<sup>th</sup> and 10<sup>th</sup>) and become dark (dark brown or black) in the remaining flagellomeres; 3) the body length: in *M. mandibularis*, specimens are variable in size (2.4–3.2 mm), whereas in *M. idreesae* is 2.5–2.9 mm.

*Microplitis mandibularis* has been reported in 19 countries from the Palaearctic and one country (Greenland) from the Nearctic region (Fernandez-Triana et al. 2020). A black/white line drawing of the T1 is available in Nixon 1970 (fig. 1, p. 6).

***Microplitis idreesae* and *M. spectabilis*.** In both species the wings are often almost uniformly hyaline; the scutellum becoming strongly shining over most of its median surface and only vaguely sculptured; on the hind wing, the vannal lobe is small; the hind tibia without apical infuscation; gregarious lifestyle; the cocoon is oval, lacking any kind of remarkable ornament, the silk fibers look disordered and fluffy, the body length in *M. spectabilis* ranges between 2.6 to 2.8 mm, and in *M. idreesae* is between 2.5 to 2.9 mm; and setae of the metasoma somewhat inconspicuous, often restricted to a single row on the tergites.

The two species can be separated by the following characters: 1) colour on the tegula: in *M. spectabilis* is yellow, whereas in *M. idreesae* is dark yellow-brown, 2) antennal flagellomeres length: in *M. spectabilis* are rather thick and somewhat smooth looking towards apex, whereas in *M. idreesae* are longer than wider and the pubescence are present along its entire surface; 3) length of the penultimate antennal flagellomere: in *M. spectabilis* it varies from one and one third to one and a half times longer than wide, whereas in *M. idreesae* is at least 2 times longer than wide, 4) antennal flagellomeres in the males: in *M. spectabilis* they are apparently always at least slightly paler beneath,

whereas in *M. idreesae* the pale colouration (yellow-brown) is clear in the first eight proximal antennal flagellomeres and gets gradually darker in the next two flagellomeres (9<sup>th</sup> and 10<sup>th</sup>) and become dark (dark brown or black) in the remaining flagellomeres; 4) apex of the hind tibia: in *M. spectabilis*, seen from the side the hind tibia is a little broaden before apex, whereas in *M. idreesae* the apex is not broaden, and 5) position of the r vein concerning the pterostigma: in *M. spectabilis* the r is perpendicular to the pterostigma, whereas in *M. idreesae* the r is oblique to the pterostigma.

*Microplitis spectabilis* has been reported in 36 countries from the Palaearctic region and there is also been recorded in the Oriental region (Pakistan) (Fernandez-Triana et al. 2020). Black/white line drawings of some structures are available in Nixon 1970 (fig. 11, p. 9; fig. 25, p. 11) and Papp 1984 (figs 64–67, p. 123).

**Comments.** As mentioned before, in 2017 one morpho-species was reported in the KSA (Fernandez-Triana and van Achterberg 2017), but the only data associated were morpho-species number (*Microplitis* sp. 6), numbers of females and males (2 females, 1 male), the collecting date (only the year, 1959), and the collecting site (Riyadh, the administrative center of Riyadh Province, located in the center of the country). Specimens are old, and not in particularly look conditions, without molecular data, and are currently deposited in the Canadian National Collection of Insects (CNC), Ottawa, Ontario, Canada (Fernandez-Triana, pers. commun.).

## Discussion

### *Microplitis* from the KSA

The Kingdom of Saudi Arabia is located at the intersection of three biogeographic regions, Palaearctic, Afrotropical, and Oriental. The previous three *Microplitis* species reported were caught in the southwestern part of the country. This is a mountainous region that runs parallel to the Red Sea, includes areas near the Yemeni border, and consists of mountains, plains, and valleys. The area is divided by steep rocky mountains into two distinct topographical zones: Tihama a lowland coastal plain at the west, and the Asir mountains range at the east (Ghramh et al. 2020). On the other hand, the new species, reported here, was collected in Hofuf, which is located on the opposite side of the country at least 1,000 km to the northeast, and is characterized by a hot desert climate.

Some tropical microgastrine genera (e.g., *Beyarslania* Koçak & Kemal, *Miropotes* Nixon, *Venanides* Mason, *Wilkinsonellus* Mason) have been reported from the southwestern part of the Arabian Peninsula (mainly Yemen), showing a clear faunal similarity with the Afrotropical region (Fernandez-Triana and van Achterberg 2017). This pattern has been also revealed in other groups of insects (e.g., Diptera, Lepidoptera, and Hymenoptera mainly Formicidae) (D'entrèves and Roggero 2004, Alahmed et al. 2010, Sharaf et al. 2014). In contrast, the fauna from the southeastern part of the Arabian Peninsula (Oman and UAE) collected in temperate climate localities show

more affinity with the Palearctic region (Fernandez-Triana and van Achterberg 2017). On account of that, *M. faifaicus*, *M. khamisicus*, and *M. tihamicus* likely have a close affinity with the fauna of the Afrotropical region (Table 1) whereas *M. idreesae*, the new species here described, would have more affinity with the Palaearctic fauna. Furthermore, the accidental discovery of a new species without mass sampling from the field may indicate the presence of several new species of agricultural importance. *Microplitis*, as well as the status of the insect biodiversity of the eastern region of the KSA, is yet scarcely known and it is a niche to be explored by taxonomists. It is expected that the diversity of *Microplitis*, as well as Microgastrinae for the entire country, will certainly be much higher when more material becomes available for study.

### Gene sequence data

The first DNA sequence data for a *Microplitis* species from the KSA is presented here. A fragment of the mitochondrial Cyt-b gene (381 bp) was obtained instead of the traditional standardized portion of the mitochondrial Cytochrome Oxidase I (COI) gene. Several attempts were made to amplify the COI following the standard protocols and primers but without success, for unknown reasons. Increasing the coverage of the gene sequence data library for the region would allow for comparisons with other regions, which already have genetic information of specimens and species of Microgastrinae (e.g., Smith et al. 2013).

### *Mythimna* Ochsenheimer

This moth caterpillar genus is commonly known as armyworm. Their name refers to the habit of spreading in a line across a lawn or pasture, and marching slowly forward, consuming the foliage they encounter on their path. The genus *Mythimna* was also known as *Cirphis* or *Pseudaletia* previously.

As aforementioned, the usual lepidopteran hosts for *Microplitis* are larvae from Noc-tuoidea and Bombycoidea, both superfamilies belong to Macrolepidoptera. This is a traditional term used to refer to butterflies and moths which tend to exhibit large body sizes (Mason 1981), but it is an artificial group. In both superfamilies, the caterpillars are large and live fully exposed to vegetation throughout their larval stages (Shaw and Huddleston 1991). Those characteristics make them more suited to support gregariousness, a lifestyle that is much more expressed in *Microplitis* (Fernandez-Triana et al. 2020).

Before the rearing reported here, three species of *Microplitis* had been reported parasitizing *Mythimna*. *Microplitis leucaniae* (Xu & He), a species presents in the Palaearctic and Oriental regions (Fernandez-Triana et al. 2020), had been associated parasitizing *Mythimna separata* (Walker) (Xu and He 2002; Ranjith et al. 2015). As for the Middle East, *Microplitis eremitus* Reinhard (from Iran and Turkey) parasitizes *Mythimna unipuncta* (Haworth), *M. mediator* (Haliday) (from Iran and Turkey) parasitizes *M. straminea* (Treitschke), and an undetermined species of *Microplitis* (from Iran) parasitizes *M. loreyi* (Whitfield et al. 2022).

## Biological control

Armyworms are pests of rice, corn, and other agricultural products and have an economic impact on farmers. Using insecticides to control armyworms is becoming less popular due to health-related concerns and due to the risk of the development of resistant strains (Zhao et al. 2018). Accordingly, employing natural enemies such as the currently identified parasitoid is a green solution that reduced the environmental contamination of pesticides. *Microplitis idreesae* hence can act as a biological control agent to combat moth caterpillars that are considered pests on local crops in the eastern region of the KSA. All known microgastrines are obligate endoparasitoids of larval Lepidoptera and practically every higher taxon of Lepidoptera is used as a host, making the subfamily one of the principal groups of natural enemies of caterpillars feeding on plants (Whitfield et al. 2018). Nonetheless, using natural enemies as agents of biological control requires wider identification of local species that can thrive in ambient conditions and at the same time serve candidates for mass production in the labs.

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